

Claims

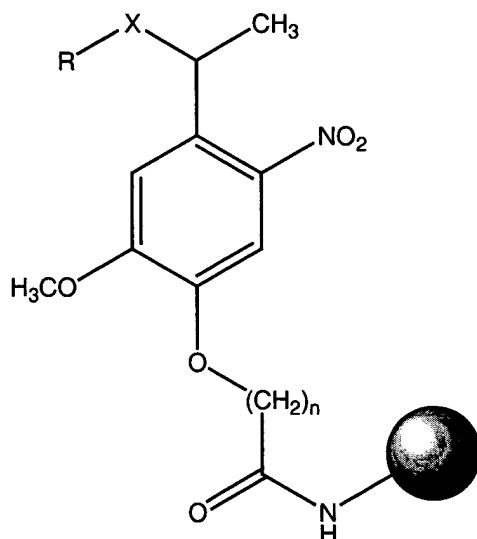
What is claimed is:

- 5 1. A method of separating phosphorylated peptides from a mixture comprising phosphorylated peptides and unphosphorylated peptides, comprising the steps of:
 - a) reacting a collection of peptides with a first resin, wherein some of
the peptides have one or more phosphate group;
 - 10 b) optionally selectively cleaving first resin that reacted with a phosphate group of the phosphorylated peptides to regenerate the phosphate group, thereby forming a resin bound collection of peptides wherein some of the peptides have one or more phosphate group;
 - 15 c) reacting the phosphate groups of the resin bound collection of peptides with a capture ligand to form a bond between the phosphorylated peptides and the capture ligand; and
 - d) separating peptides bound to the capture ligand from peptides that
are not bound to the capture ligand, thereby separating
20 phosphorylated peptides from unphosphorylated peptides.
2. The method of Claim 1, further comprising the step of selectively cleaving
the first resin before separating the peptides bound to the capture ligand,
thereby forming a second collection of peptides comprising
25 unphosphorylated peptides that are not bound to a resin and phosphorylated peptides that are bound to the capture ligand.
3. The method of Claim 2, further comprising the step of cleaving the bond
between the capture ligand and the phosphorylated peptides separated
30 in step d) to form unbound phosphorylated peptides.
4. The method of Claim 1, further comprising the step of reacting the

peptides with a reagent for protecting amine groups before reacting the peptides with the first resin.

5. The method of Claim 4, wherein the first resin comprises primary or secondary amine groups that reacts with the carboxylic acid groups of the peptides to form an amide bond and the phosphate groups of the peptide to form a phosphoramidate bond.
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6. The method of Claim 5, wherein the phosphoramidate bonds are selectively cleaved by contacting the resin bound peptides with a weak acid or a weak base.
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7. The method of Claim 5, wherein the first resin comprises a peptide or an amino acid residue bound to the first resin, wherein the peptide or amino acid residue has a primary or secondary amine group.
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8. The method of Claim 7, wherein the bound peptide or amino acid residue is isotope labeled, and wherein the isotope labeled peptide or amino acid residue remains bound to the peptide when the first resin is selectively cleaved.
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9. The method of Claim 5, wherein the first resin comprises a linker bound to the first resin, wherein the linker has a primary or secondary amine group, or a hydroxy group.
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10. The method of Claim 9, wherein the bound linker is isotope labeled, and wherein the isotope labeled linker, or a portion thereof, remains bound to the peptide when the first resin is selectively cleaved.
11. The method of Claim 2, wherein the first resin is selectively cleaved by exposing the resin bound peptides to light.
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12. The method of Claim 11, wherein the first resin is a photocleavable resin having a plurality of groups represented by the following structural formula:



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wherein:

n is an integer;

X is $-O-$ or $-NH-$;

- 10 R is $-H$, an amino acid, a peptide, a linker, an isotope labeled amino acid, an isotope labeled peptide, or an isotope labeled linker; and the shaded circle represents a resin.

13. The method of Claim 2, wherein the capture ligand is a second resin.

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14. The method of Claim 13, wherein the second resin has primary or secondary amine groups that react with the phosphate groups of the peptide to form phosphoramidate bonds.

- 20 15. The method of Claim 14, wherein the second resin comprises an amino acid residue bound to the second resin, wherein the amino acid residue has a primary or secondary amine group.

16. The method of Claim 13, wherein the second resin is magnetic and the first resin is non-magnetic, and the peptides bound to the second resin are separated by exposing the resin bound peptides to a magnetic field.
- 5 17. The method of Claim 2, wherein the capture ligand is a first recognition entity of a molecular recognition system.
18. The method of Claim 17, further comprising contacting the second collection of peptides with an affinity resin, wherein the affinity resin
10 comprise a second recognition entity of the molecular recognition system bound to a solid support, thereby binding the peptides bound to the first recognition entity to the affinity resin.
19. The method of Claim 18, wherein the molecular recognition system
15 comprises an antigen/antibody, an antigen/antibody fragment, an avidin/biotin, a streptavidin/biotin, a protein A/I_g or a lectin/carbohydrate.
20. The method of Claim 18, wherein the affinity resin is collected by filtration, thereby separating phosphorylated peptides from
20 unphosphorylated peptides.
21. The method of Claim 18, wherein the second collection of peptides is passed through a column comprising the affinity resin, thereby separating phosphorylated peptides from unphosphorylated peptides.
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22. A composition, comprising a peptide bound to a capture ligand via a phosphoramidate bond.
23. The composition of Claim 22, wherein the peptide is isotope labeled.
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24. The composition of Claim 22, wherein the peptide is bound to a first resin that can be cleaved from the peptide under conditions wherein the bond

between the capture ligand and the peptide is stable.

25. The composition of Claim 24, wherein the peptide is isotope labeled.

5 26. The composition of Claim 24, wherein the first resin can be cleaved from the peptide by exposure to light.

27. The composition of Claim 24, wherein the capture ligand is a second resin.

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28. The composition of Claim 24, wherein the capture ligand is a first recognition entity of a molecular recognition system.

15 29. The composition of Claim 28, wherein the first recognition entity is selected from the group consisting of an antigen, an antibody, an antibody fragment, an avidin, a biotin, a streptavidin, a protein A, a lectin, and a carbohydrate.

20 30. A kit for separating phosphorylated peptides from a mixture comprising phosphorylated peptides and unphosphorylated peptides, comprising:
a) a first resin that can react with a carboxylic acid group of a peptide to form a covalent bond; and
b) a capture ligand that can react with a phosphate group of a peptide to form a covalent bond, wherein the first resin can be
25 cleaved from the peptide under conditions wherein the bond between the capture ligand and the peptide is stable.

31. The kit of Claim 30, further comprising a reagent for protecting amine groups.

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32. The kit of Claim 30, further comprising a reagent for cleaving the bond between the phosphate group of the peptide and the capture ligand to

regenerate the phosphate group.

33. The kit of Claim 30, wherein the capture ligand is a second resin.
- 5 34. The kit of Claim 30, wherein the capture ligand is a first recognition entity of a recognition system.
35. The kit of Claim 34, further comprising an affinity resin comprising a second recognition entity of the molecular recognition system bound to a solid support.
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36. The kit of Claim 35, wherein the molecular recognition system comprises an antigen/antibody, an antigen/antibody fragment, an avidin/biotin, a streptavidin/biotin, a protein A/I_g or a lectin/carbohydrate.
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37. The kit of Claim 30, wherein the first resin comprises an isotope labeled linker, an isotope labeled amino acid, or an isotope labeled peptide.